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14. ABSTRACT
The purpose of the research is to analyze the expression and function of gp140/CDCP1, a regulator of SRC kinase activity in prostate cancer. The scope of the project in year 3 is to determine expression in tissues from patients. The main results are from immunohistochemical analyses of gp140 in normal prostate epithelium, primary prostate cancer and metastatic prostate cancer. Gp140 RNA expression and protein membrane expression, but not total protein expression, are significantly decreased in primary cancers compared to normal epithelium. In metastatic disease, total gp140 protein expression decreases and is significantly lower in bone compared to soft tissue metastases. Overall, gp140 expression decreases during metastatic progression, supporting the concept that the loss of gp140 facilitates tumor metastasis.

15. SUBJECT TERMS
prostate cancer, gp140/CDCP1, immunohistochemistry, tumor metastasis

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Annual Report for
KNUDSEN Lab, Cedars Sinai Hospital
(May 1, 2011 to April 30, 2012)

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INTRODUCTION:

SUBJECT, PURPOSE, SCOPE -

Subject: The central hypothesis of this project is that loss of Gp140 expression promotes invasion and metastases in prostate cancer cells. Gp140 was discovered in the Carter lab in 1996, but little was known about its function until 2004 when it was discovered to be a substrate of Src kinases and a binding protein of PKCδ [1] [2-4]. More recently, several studies elucidated additional functions of Gp140/CDCP1, such as expression in stem cells [5], anoikis [6, 7], migration [8] and regulation of subcellular Src activity [9-11]. Furthermore, based on recent studies, the activity of Gp140/CDCP1 may be cancer type and context dependent as CDCP1 was noted to suppress [12, 13] as well as enhance tumor progression and metastasis [14-16]. Finally, an antibody against gp140/CDCP1 inhibited metastases in a subcutaneous xenograft model of PC3 cells [17]. Together these data highlight the important role of Gp140/CDCP1 in prostate cancer and warrant specific studies in prostate cancer to determine CDCP1's role in this cancer type.

The purpose of the research is to analyze the role of gp140/CDCP1 in prostate cancer metastasis and to determine whether targeting gp140/CDCP1 could serve as a treatment against metastatic prostate cancer.

The scope of the research includes experiments in cultured cells, in cell line xenografts and in human tissues.

BODY:

RESEARCH ACCOMPLISHMENTS –
With the activation of the grant at Cedars Sinai in the fall of 2011, Dr. Knudsen started to organize and plan experiments outlined in Task 3 and Task 4 of the statement of work.

"***Task 3 (months 3-9, Dr. Knudsen):***<u>To investigate whether Gp140 regulates Tyr-phosphorylation of the AR by SFKs.</u>

- We will measure changes in nuclear localization and Tyr-phosphorylation of AR after inducing phosphorylation of Gp140.
- We will analyze changes in AR activity after Gp140 phosphorylation using a luciferase reporter construct

The milestone at the end of this task is to evaluate using quantitative data whether or not phosphorylation of Gp140 regulates Tyr-phosphorylation of the AR.

Task 4 (months 6 – 24, Dr. Knudsen):<u>To determinewhether the expression level of Gp140 and of a Gp140 biomarker panel predicts the development of prostate cancer metastasis."</u>

To accomplish task 3 of the grant, Dr. Knudsen recruited a senior project scientist to the lab. Dr. Nishit Mukhopadhyay has extensive expertise in signal transduction, kinases and AR. He will determine whether CDCP1 regulates the phosphorylation of the AR by Src kinases. To this point, he has received antibodies from Dr. Carter to "activate" CDCP1 in prostate cancer cells.

To complete task 4, we established conditions for the CDCP1 antibody in formalin fixed and paraffin embedded (FFPE) tissues. We have hired a histotechnician and are optimizing the antibody in the laboratory at Cedars Sinai. In addition, we are obtaining clinical follow-up on 330 prostate cancer cases in the biobank at Cedars Sinai and will use this cohort to determine the association of CDCP1 expression and prostate cancer progression. We also established a state-of-the-art digital image analysis core and will be able to obtain more accurate, reproducible and sensitive expression measurements. Altogether, we have enhanced our resources to complete the research proposed in task 4.

REPORTABLE OUTCOMES - none

CONCLUSIONS:

With considerable leverage from the institution, we have established the infrastructure to pursue the remaining research that we proposed in task 3 and task 4 of the award. As of November 1st 2012, we hired the personnel to continue to work on the role of CDCP1 in prostate cancer metastases and will complete the research in a timely manner and publish the results.

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